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Aminoglycosides—50 years on

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1 The aminoglycoside antibiotics are 50 years old. Their success and continuing use can be attributed to various factors including rapid concentration-dependent bactericidal effect, synergism with β-lactam antibiotics, clinical effectiveness, a low rate of true resistance and low cost.
2 The aminoglycosides remain drugs of choice in many circumstances including septicaemia, other serious infections due to Gram negative bacilli, and bacterial endocarditis.
3 Nephrotoxicity and ototoxicity have been the main drawbacks clinically for the aminoglycosides.
4 There has been an evolution in dosing strategies largely aimed at reducing toxicity. Therapeutic drug monitoring has been used extensively to assist dosing, and target concentrations have been advocated, such as peak concentrations of between 6 and 10 mg l^{-1} and trough concentrations of < 2 mg l^{-1} for gentamicin, tobramycin and netilmicin.
5 Recently there has been a minor revolution in the approach to aminoglycoside dosing, with a change to larger doses, given less frequently. In its most convenient form this is 'Once-daily aminoglycoside dosing'. It offers the hope of better efficacy, less toxicity, and easier administration and monitoring.
6 This article summarises the background of aminoglycoside usage, leading up to the recent changes in dosing strategy.

Keywords aminoglycosides history dosing

Introduction

The first aminoglycoside, streptomycin, was introduced in 1944. It was isolated from a strain of *Streptomyces griseus* in a well-planned search for antibacterial substances, stimulated by the discovery of penicillin [1]. In 1949 neomycin was isolated from *Streptomyces fradiae* [2], followed by kanamycin from *Streptomyces kanamyceticus* in 1957 [3]. Gentamicin was isolated from the actinomycete *Micromonospora purpurea* in 1963 [4], the 'micin' spelling reflecting the different species of origin. Netilmicin, introduced in 1976, is a semisynthetic derivative of sisomicin which also comes from Micromonospora species [5]. Tobramycin was produced from *Streptomyces tenebrarius* in 1967 [6] and amikacin, a semisynthetic derivative of kanamycin, was introduced in 1972 [7].

The aminoglycosides consist of two or more amino sugars (aminoglycosides) connected to an aminocyclitol nucleus. They should strictly be called aminoglycoside-aminocyclitols but this has been abbreviated for simplicity. The different aminoglycosides are distinguished by their amino sugars. Gentamicin, as used therapeutically, is a group of three structurally similar variants. Gentamicin, tobramycin, netilmicin and amikacin are the main aminoglycosides in current use for major sepsis while streptomycin retains a place in the treatment of tuberculosis.

Mechanism of action

Aminoglycosides are bactericidal as a result of inhibition of protein synthesis and altered integrity of the bacterial cell membrane [8]. In growing bacteria, after diffusion through the outer membrane, there is low affinity binding of the aminoglycoside to an energy-dependent transport system termed 'phase I transport', which enables uptake across the inner (cytoplasmic) membrane. This step is rate-limiting and can be blocked by calcium and magnesium ions,

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hyperosmolarity, low pH and anaerobic conditions. Sensitive bacteria accumulate the drug intracellularly at a site of high affinity on the 30S subunit of ribosomes associated with the cell membrane. This triggers a second energy-dependent transport system termed 'phase II transport', resulting in marked acceleration of intracellular accumulation of drug. Inhibition of protein synthesis and disruption of the structure of the cytoplasmic membrane follows. Leakage of intracellular contents precedes cell death [9]. The extremely rapid kill-rate of the aminoglycoside suggests that lethal events occur prior to the disruption of protein synthesis. Gentamicin has been shown to destabilise the outer membrane of *Pseudomonas aeruginosa* and form holes in the cell wall, independent of its action on ribosomes [8]. This action of the aminoglycosides may be the most important.

Mechanism of true resistance

Resistance to aminoglycosides is largely related to impaired transport into microbes [10]. Plasmids in the cytoplasm produce inactivating enzymes and resistance factors which prevent aminoglycoside binding to phase II transport proteins. Deactivating enzymes include phosphorylases, adenylylases and acetylases, which act on hydroxyl or amino groups of the aminoglycosides.

Adverse reactions

Nephrotoxicity and ototoxicity are the most important adverse effects clinically, and have dominated attempts to rationalise aminoglycoside dosing [11].

Nephrotoxicity

The major site of damage is the proximal renal tubule. Uptake of aminoglycoside into tubular cells is via calcium-dependent active transport, which is saturable for gentamicin and netilmicin at concentrations attained clinically [12]. Amikacin uptake is saturable to some extent, but studies have been unable to demonstrate tobramycin saturability [12]. The exact chain of events in aminoglycoside nephrotoxicity is unclear, but accumulation of drug and phospholipids within lysosomes is involved. The lysosomes become overloaded with phospholipid, destabilise and rupture, releasing acid hydrolases and high concentrations of aminoglycoside into the cytoplasm where they disrupt cell structure and function [13]. The toxic potential of individual aminoglycosides is directly related to their capacity to bind to and perturb membrane function, and is reflected in the degree of phospholipiduria, an early index of nephrotoxicity [14]. Results from clinical trials and animal studies suggest the following rank order of decreasing nephrotoxicity using equitherapeutic doses, although the middle four drugs may differ only marginally: neomycin > gentamicin ≥ tobramycin ≥ amikacin ≥ netilmicin > streptomycin [13, 15–17].

Clinically, nephrotoxicity is expressed as non-oliguric renal failure, with varying degrees of tubular dysfunction [18, 19]. Glomerular filtration rate decreases as a relatively late event, usually at least 5–7 days after initiation of therapy [20]. Renal function recovers completely in most patients [21].

Risk factors that are consistently reported for nephrotoxicity include choice of aminoglycoside, prolonged duration of therapy, greater total aminoglycoside dose, hypotension, volume depletion, high peak or trough serum concentrations, concurrent liver disease, and concurrent use of other nephrotoxic drugs [15, 22–24]. Pre-existing renal impairment and older age have been identified as risk factors, but the association is likely to relate to inappropriate dosing in the presence of diminished renal function [13, 15, 25].

Ototoxicity

Ototoxicity was discovered in the first clinical trial of streptomycin in 1945 [26]. Tinnitus is a frequent early symptom. Hearing loss occurs as a result of degeneration of the hair cells of the cochlear, beginning at the basal coil and progressing to the apex. High frequency hearing loss is followed by loss of lower frequencies. By the time hearing loss is reported clinically, substantial damage has already occurred [27].

Within the vestibular apparatus, hair cell damage starts in the apex of the cristae and the striolar regions of the maculae and progresses towards the periphery of the vestibular receptor [28]. Along with the sensory cells, afferent nerve endings deteriorate. Vestibulotoxicity presents clinically as disequilibrium and ataxia.

Both acute and chronic ototoxicity have been observed. The acute type is reversible, while the chronic type may be largely irreversible. The exact mechanisms involved and the relation to the dose and the dosing regimen of aminoglycosides remain unclear, although there are some parallels with the effects in the kidneys: acute reversible hearing loss may relate to competitive antagonism between the drug and calcium [29]; chronic toxicity may relate to aminoglycoside-phosphoinositol binding leading to altered membrane structure and permeability [30, 31].

Streptomycin is predominantly vestibulotoxic, while amikacin appears to be exclusively cochleotoxic [32]. Gentamicin, tobramycin and netilmicin may affect either system [33]. There is some variation between clinical trial results but a rank order of decreasing cochlear toxicity with equitherapeutic doses may be: neomycin > amikacin = kanamycin > tobramycin = gentamicin = streptomycin > netilmicin [34–41]. Similarly, a rank order of decreasing vestibular toxicity may be: streptomycin > gentamicin > tobramycin = kanamycin = amikacin = neomycin > netilmicin [35–37].

Risk factors have been difficult to isolate owing to the difficulties in studying ototoxicity. No definite risk factors can be cited, although many have been proposed [36]. Chronic toxicity is unpredictable, sometimes with sudden and severe onset, and may relate to the area under the concentration-time curve (AUC) and the total dose of aminoglycoside given [42]. There

is some evidence for genetic predisposition to ototoxicity [43, 44].

Dosing strategies

It is salutary to note that the dose recommendations for gentamicin in the fourth edition of Goodman & Gilman [45] were 0.8–1.2 mg kg^{-1} day^{-1} given in two to four divided doses for patients with normal renal function.

More sophisticated dosing strategies arose out of a desire to minimise toxicity. Early studies suggested that the incidence of toxicity increased with serum peak concentrations > 12 mg l^{-1} or trough concentrations > 2 mg l^{-1}, for gentamicin or tobramycin [46–48].

Several studies have suggested that patient mortality is reduced in serious infections if peak concentrations are above 5, 6 or 7 mg l^{-1} early in the course of treatment [49–51].

Studies on efficacy and toxicity have led to the recommendation that serum peak concentrations should be 6–10 mg l^{-1} for gentamicin and tobramycin (and probably netilmicin), and 20–40 mg l^{-1} for amikacin. Recommended trough concentrations were < 2 mg l^{-1} for gentamicin and tobramycin (and netilmicin) or < 7 mg l^{-1} for amikacin [52–54]. Dosing strategies designed to achieve these target concentrations were initially based on nomograms which estimated dose requirements on the basis of renal function. Aminoglycosides are eliminated largely unchanged by the kidneys, and maintenance dose requirements were thought to correlate well with indices of renal function such as creatinine clearance [55].

The recognition of large variability in the pharmacokinetics of aminoglycosides led to attempts to individualise dosing based on measurement of the patient's own aminoglycoside clearance (CL) and volume of distribution (V). A pharmacokinetic model based on the assumptions of a single compartment and first-order elimination was proposed by Sawchuk *et al.* [56]. This became known as the Sawchuk-Zaske method. After infusion (usually over 30 min) of a standard dose of aminoglycoside, at least three blood samples are drawn over increasing time intervals. The serum concentration-time data are fitted to a single exponential term using linear regression, allowing the patient's own V and CL to be calculated. The values for these parameters are then used to calculate a dose and dose interval to achieve desired peak and trough concentrations. Others derived similar dosage strategies [57].

Another interesting method of individualising aminoglycoside dosing uses a statistical approach based on Bayes' theorem [58, 59]. Applied to the estimation of the pharmacokinetic parameters of a drug, Bayes' theorem describes quantitatively the relationship between the probability of a patient having certain values for pharmacokinetic parameters 'before' any serum concentration data are available for that patient (prior probability), and the subsequent probability of having these values 'after' measured drug concentrations are available (posterior probability). With respect to the aminoglycosides, the first dose is estimated from values of V and CL based on all information known about the patient and the population in general, taking into account measurement errors. As sequential concentration-time data become available, the values of the parameters reflect more closely the patient's 'real' values and less those of the population.

Performance of the different dosing methods

Nomograms perform poorly despite being an improvement on fixed dose schedules. Zaske *et al.* [60] found a large variation in pharmacokinetic parameters in patients with normal serum creatinine or estimated creatinine clearance and postulated that nomograms would be expected to perform badly. Failure to achieve adequate peak serum concentrations in the majority of patients has been a major problem [61–65].

The large variability in pharmacokinetic values has been confirmed in many studies. Severely ill patients often have an increased V with considerable interpatient variation, and the V appears to return towards normal as the sepsis improves [66–69]. Further, the correlation between serum aminoglycoside CL and creatinine clearance is not sufficiently good in ill patients to allow accurate prediction of aminoglycoside CL [70, 71]. Errors of several hundred percent may result if aminoglycoside dosing is predicted from creatinine clearance [72].

Individualised pharmacokinetic approaches, exemplified by the Sawchuk-Zaske method, understandably are superior to nomograms or empirical methods at achieving target concentrations. This has been confirmed in various trials, both retrospective [62, 73] and prospective [74, 75].

Dosing using Bayesian methods is also superior to nomogram or empirical methods at achieving target concentrations [76]. Various studies have retrospectively compared Bayesian methods with the Sawchuk-Zaske method [77–79]. While minor differences were evident, the two methods were difficult to distinguish in overall performance, although the Bayesian method was the most efficient.

The Sawchuk-Zaske and Bayesian methods have been well shown to achieve target concentrations more accurately than older methods but there have been few, if any, studies which show that this translates to improvement in morbidity or mortality. Early studies suggested that the use of nomograms was accompanied by enhanced eradication of sepsis [80], or fever [81], and circumstantial evidence [49–51] would suggest that the Sawchuk-Zaske and Bayesian methods should be superior because of the achievement of higher peak concentrations.

Target concentrations revisited

While the ability to achieve target concentrations was improving, many investigators turned their attention to questioning the targets themselves. Are peaks of 6–10 mg l^{-1} and troughs of < 2 mg l^{-1} the be all and end all? There are many reasons why they are not ideal targets.

Several pharmacodynamic features of the aminoglycosides favour the administration of larger doses given less frequently than with conventional therapy. There is convincing *in vitro*, animal and human clinical data which supports this rationale. Aminoglycosides, unlike β-lactam antibiotics, display concentration-dependent bacterial killing both *in vitro* and *in vivo* [82–84]. Expressed simply, the higher the concentration, the greater the kill.

The postantibiotic effect (PAE) refers to continued suppression of bacterial growth after antibiotic concentrations have dropped below the minimal inhibitory concentration (MIC). For the aminoglycosides, a PAE of 1–8 h has been shown both *in vitro* and *in vivo* for various Gram-negative bacilli after exposure to drug at concentrations 2–10 times the MIC [85, 86]. When higher doses of aminoglycoside are used the PAE is even longer [84, 87]. The PAE allows a longer dose interval than predicted based on the MIC alone.

Adaptive resistance is a recently recognised phenomenon that describes reversible refractoriness to the bactericidal action of aminoglycosides [88, 89]. The phenomenon was observed in several *in vitro* studies in which second and subsequent doses of aminoglycoside had diminished or no bactericidal effect [90, 91]. It has been shown with all aminoglycosides and in all Gram-negative bacilli studied.

The mechanism of adaptive resistance to aminoglycosides seems to relate to down-regulation of the phase II energy-dependent uptake of the drug into bacteria [88, 92]. Adaptive resistance occurs within 2 h of drug exposure both *in vitro*, and *in vivo* (mice) [88]. The time-course for return of susceptibility to the aminoglycoside depends on the model and the bacterium. The major correlate appears to be the time course of removal of the aminoglycoside from the site of the infection. *In vitro* studies in which the aminoglycoside is rapidly washed out of growth media have shown that adaptive resistance lasts about 6–7 h [88, 92]. In mice, in which the half-life of aminoglycosides is a very short (around 15 min), adaptive resistance persists for around 8 h [88]. In a dynamic *in vitro* model simulating human aminoglycoside pharmacokinetics, adaptive resistance in *Pseudomonas aeruginosa* is maximal for up to 16 h with full return to susceptibility as late as 40–44 h after a single dose of gentamicin [92]. Adaptive resistance is likely to persist even longer in peripheral compartments, and in patients with longer half-lives such as in renal impairment. The extent of adaptive resistance should not be underestimated. Conditioned organisms continue to grow in concentrations as high as 128 times the original MIC of the drug [93].

In relation to efficacy, there are thus strong arguments in favour of using larger doses given less frequently. There is also a compelling case based on toxicity considerations.

As noted, nephrotoxicity appears to relate to the amount of drug that accumulates in the renal tubular cells. The uptake of aminoglycoside into the cortex appears to be saturable at concentrations achieved clinically, at least for gentamicin, netilmicin and to some extent amikacin. Saturability means that a lower percentage of the total dose administered will get into the renal tubular cell, if the drug is given in larger doses less frequently. The situation with ototoxicity is less clear, although there is some evidence supporting saturable uptake into the sites of damage [94].

There are thus good theoretical reasons to support the use of larger doses given over longer dose intervals than is conventional practice.

Clinical studies

There have been at least 29 studies in humans comparing once-daily aminoglycoside administration with conventional, more frequent administration [95]. Around 22 have failed to show any difference in efficacy or toxicity. Seven studies have demonstrated a difference in favour of once-daily dosing. One study demonstrated better efficacy in the once-daily group [96]. Of six studies demonstrating less toxicity in the once-daily group, five showed less nephrotoxicity [97–101] and two showed less ototoxicity [98, 102]. No study has shown an advantage for the conventional approach.

It is not surprising that many studies were unable to demonstrate a difference between regimens. Often rather gross measures of efficacy and toxicity were used, and in most trials only small populations were studied. Differences in efficacy are particularly difficult to demonstrate in the setting of a high response rate in the control group and with the influence of another antibiotic, usually a β-lactam, which is invariably part of the regimen. In most studies in which nephrotoxicity was monitored, only crude indices of renal dysfunction were used such as change in serum creatinine concentration or creatinine clearance. Similarly for ototoxicity, most studies involved only clinical assessment, or standard audiometry (up to 8 kHz). In only two studies was a sensitive index of nephrotoxicity (phospholipiduria) used. In both, a difference in favour of once-daily dosing was evident [97, 98]. Similarly, only three studies that monitored ototoxicity utilised high frequency audiometry (10–18 kHz), and in two of these a difference in favour of once-daily dosing was evident [97, 98, 102].

The use of once-daily dosing in the setting of neutropenia has been debated but data for this group are encouraging. There have been at least six trials in neutropenic patients, all of which have included concomitant β-lactam therapy. One trial demonstrated significantly better efficacy with once-daily administration [96]. Indeed this has been the only trial to achieve this. Two trials showed advantages of longer dose-intervals in terms of toxicity [101, 103]. Results from *in vitro* dynamic models of infection, which mimic the situation in the neutropenic host, provide good theoretical support for the use of larger doses less frequently [92].

The use of the 24 h dose interval is largely based on convenience. The optimum dose and dose interval has yet to be determined. It may be that a larger dose every 48 h is best, or a single very large dose followed by regular administration of a different antibiotic. Undoubtedly there will be differences in the optimum regimen for different bacteria and different types of infection.

Many researchers now recommend once-daily aminoglycoside therapy in selected patient groups. However, the optimal dose size and method of dose adjustment remains unclear. The use of target peak and trough concentrations is less satisfactory than with conventional dosing. Peak concentrations will always be adequate with the larger doses used. Trough concentration management will not be useful since predicted concentrations at 24 h will be unrecordable in patients with normal aminoglycoside clearance. Further, dose alteration will not be possible until the third dose, 48 h after the start of therapy, when it is arguably too late. For these reasons a new approach to monitoring and dose adjustment is necessary for once-daily dosing.

Conclusions

The aminoglycosides have come a long way since their introduction 50 years ago. They are one of the most successful drug classes in the history of modern medicine, and have survived takeover attempts from later generation penicillins, cephalosporins and the quinolones. Perhaps we are just beginning to learn how to use them properly.

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